ORIGINAL ARTICLE

HISTOMORPHOLOGICAL EFFECTS OF HUNGER STRESS ON OVARIES

Maria Yousaf, Hina Siddiqi*, Nazish Waheed*

Department of Anatomy, Rehman Medical College, Peshawar, *Department of Anatomy, Pak International Medical College, Peshawar-Pakistan

Background: Incidence of stress is on the rise in our daily life involving various neurobiological, endocrinological and behavioral changes. Hunger stress has a potent influence on mental, physical, and reproductive health by affecting the hypothalamic-pituitary gonadal axis. **Methods:** It was a laboratory based randomized control trial. Adult female mice (BALB-c strain) weighing 25–27 grams on first day of estrous cycle were taken in two groups (ten each). Group A was kept in normal environment of animal house for one month. Group B was given hunger stress by restricting the diet to about 50% per day for one month. Right ovaries of the animals were dissected out and observed for shape, color, and weight. Histological slides were prepared for the count of primary, secondary, and tertiary follicles. **Results:** Statistically significant decrease in animal and ovary weight with significant fall in ovarian follicles was observed. **Conclusion:** Hunger stress affects the ovaries by reducing its weight and number of follicles.

Keywords: Stress; Hunger; Ovarian follicles; Ovary

J Ayub Med Coll Abbottabad 2017;29(4):654–7

INTRODUCTION

Stress is increasing in our daily life involving various neurobiological, endocrinological and behavioral changes. Hunger stress has a potent influence on mental physical and reproductive health. Nutritional status influence fertility which has marked effects on the growth and development of follicles.[1]

In the event of hunger, the process of cell proliferation slows down leading to prolonged cell cycles and arresting some cells at G1 stage.[2] Prolonged exposure to stressors can trigger sympatho-adreno-medullary system and hypothalamic-pituitary-adrenal (HPA) axis. The degree of triggering depends on its type, intensity and duration. It has been suggested by many studies that stress may cause infertility by affecting the hypothalamo-pituitary gonadal axis.[3]

Body weight is highly dependent on the food intake, so hunger stress leads to weight reduction, decrease in lean mass, fat mass, serum leptin and insulin like growth factor (IGF-1).[4] Nutrition plays a fundamental role in determining fertility with hormones like testosterone production is decreased by food restriction.[5] It also influences the reproductive efficiency of cows by acting on the hypothalamic-pituitary-ovarian axis.[6]

Stress proves to be an add-on to the oxidant burden which results in damage to DNA, lipids, and proteins; and accelerates process of aging.[7] In females, chronic stress inhibits luteinizing hormone (LH) and follicular stimulating hormone (FSH) secretion leading to disturbed reproductive activity including inability to conceive, failure of embryo to implant, repeated abortion and menstrual cycle irregularities.[8]

The purpose of the study is to observe the effects of hunger stress on ovarian follicle count in a laboratory based study.

MATERIAL AND METHODS

This laboratory based randomized controlled trial was carried out in the Department of Anatomy, Army Medical College, Rawalpindi with the support of National Institute of Health (NIH). Ten adult non-pregnant female mice (BALB-C strain), 5–7 weeks old, weighing 25–27 grams were taken as control in group A and the same number as cases in group B. Assignment to both groups was done randomly after assigning them numbers. Both the groups were kept and fed with standard diet of NIH laboratory. Mice in group A were provided with the favorable environment of animal house for 1 month while mice in group B were exposed to hunger stress by limiting the diet to average 3gm of commercially pelleted food per day for 1 month which is about 50% of *ad libitum* food intake.

The animals were dissected after 1 month. Right ovary from each animal was removed to maintain uniformity and observed for its shape, color and weight. They were then kept in 10% formalin. Standardized methodology for preparation of histological slides, stained with hemotoxylin and eosin was followed. 10X objective was used to count primary (FI), secondary (FII) and tertiary follicles (FIII). They were counted starting from the mesentery which is attached to the anterior border of the ovary, moving clockwise.[9] Three slide per specimen were observed.[10]

Data was analyzed using SPSS version 20. Independent sample t test was applied to find the *p*-values. Results were considered significant with a *p*-value of <0.001.

RESULTS

Ovaries of animals in control group A appeared as round in 3 animals and ellipsoid in 7 animals, pale whitish in color and firm in consistency. No

hemorrhages and adhesions were found on the surface of the ovaries. Microscopically, all ovaries in group A were lined with simple cuboidal epithelium. Underneath the epithelium was *tunica albuginea* consisting of collagen fibers. The ovarian tissue was divided into inner medulla, which contained stroma and blood vessels and an outer cortex, which contained the follicles at different stages of development. Growing follicles were seen in considerable number among the stroma of the cortex. Single or several layers of granulosa cells surrounded the oocyte. In secondary follicles, there was small accumulation of fluid in the intracellular spaces called follicular fluid among the granulosa cells. Graafian follicles were observed with a peripheral oocyte surrounded by cumulus cells and several layers of granulosa cells with a large antrum. (Figure-1)

Ovaries of hunger stress exposed animals in group B were round in 4 animals and ellipsoid in 6 animals and were smaller in size. Color of the ovaries in 2 animals was reddish while in 8 animals was pale whitish. Histological sections showed disturbance in the germinal epithelium. Spaces or vacuoles were observed in the tunica albuginea. There was an apparent decrease in the number of primary and secondary follicles as compared to control. Graafian follicles were much reduced in number and were absent in some sections. Shape of the follicles was also distorted as there was reduction in the number of granulosa cells. (Figure-2)

The mean±SD change of body weight of animals in group A was from 25.70±0.856 to 45.05±1.21 gm while in experimental group this change was from 25.45±0.926 g to 29.75±1.84 g respectively. The *p*-values for change in body weight was <0.001 which was statistically significant. The mean±SD weight of the ovary of mice in group A was 0.0185±0.000527g while in experimental group was 0.0176±0.000516 g. The ovary weight in experimental group B was less than group A. The *p*-value was 0.002 which was statistically significant. The mean±SD number of primary follicle in group A was 12.5±2.37 while in group B it was 9.6±0.97. The *p*-value was 0.001 for primary follicles which was statistically significant.

The mean number of secondary follicle in group A was 6.70±1.42 while 4.90±0.738 in group B. The *p*-value was 0.001 for secondary follicles when compared to control group A which was statistically significant.

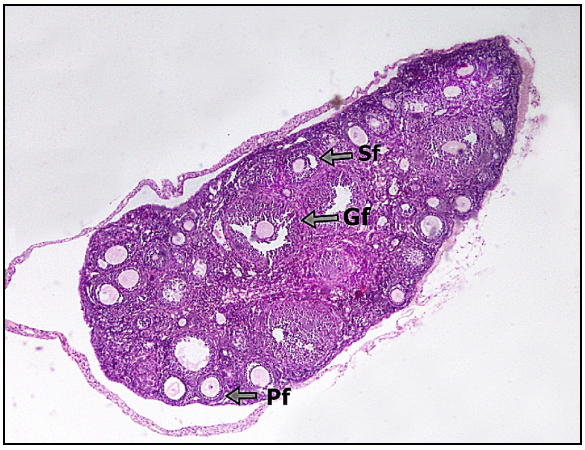

Figure-1: Photomicrograph of transverse section of ovary showing, Pf; primary follicle, Sf; secondary follicle and Gf; Graafian follicle (X600) in control group A.

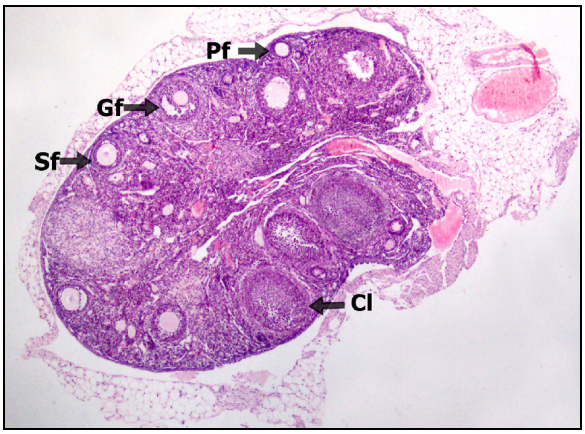

Figure-2: Photomicrograph of transverse section of ovary showing fewer number of follicles in experimental group B. Pf; primary follicle, Sf; secondary follicle, Gf; Graafian follicle, Cl; corpus luteum. (600 X)

Table-1: Count of ovarian follicles with diameter of Graffian Follicle in control group A and experimental group B

Animal No.	Primary Follicle (FI)		Secondary Follicle (FII)		Graafian Follicle (FIII)		Diameter of Graffian follicle(µm)	
	Control	Experimental	Control	Experimental	Control	Experimental	Control	Experimental
1	18	9	5	5	2	1	302.5	290.0
2	14	8	7	4	3	1	355.0	298.0
3	13	9	5	5	2	1	322.5	285.0
4	10	10	8	5	0	0	NA	NA
5	11	11	6	4	2	2	325.0	298.0
6	11	10	9	5	1	1	330.0	290.0
7	10	9	5	6	3	1	345.0	288.0
8	12	11	7	5	3	1	310.0	292.0
9	13	9	8	4	2	1	320.0	284.0
10	13	10	7	6	3	1	325.0	298.0

DISCUSSION

Stressful situations lead to many psychological and physiological changes in equilibrium or homeostasis and are a continuous threat to a normal health and well-being.

In the present study, the mean change of body weight was statistically significant when control group A was compared with experimental group B (<0.001). Prior studies established the relationship between hunger and the skeleton and showed that rodents exposed to hunger stress had decreased weight gain due to reduced skeletal growth, cortical and spongy bone mass and bone loss.[4]

Statistically significant difference was observed when the mean value of ovary weight was compared among the groups. Reduction in the ovarian weight of stressed rats specifies the decrease in activity of stroma, growing follicles and corpus luteum due to non-availability of steroidal or gonadotrophic hormones or both.[11]

Various experimental studies revealed that different stressors of 6 hours per day duration to 15 days old rat pups affected ovarian follicular development resulting in marked reduction of primordial, primary and pre-antral follicles.[12] The present study also supports this as the difference in number of primary follicle was statistically significant between the control group A and experimental group B.

A statistically significant difference in the number of secondary follicles was observed when group A was compared with group B supporting previous studies where reduction in number of secondary follicles was observed in rats exposed to heat and cold stress.[13,14]

Pre-antral and antral follicles have LH and FSH receptors and the fluctuation in the gonadotrophin levels due to stress causes degenerative changes in the developing antral follicles.[15]Various hormones within the hypothalamus-pituitary-ovarian axis are under the influence of food intake and control ovarian activity. It has been proved that diet has a positive effect on the growth rate and size of the ovulatory follicle further supported by present study with statistically significant difference in the number and size of Graafian follicles in Group A and B.[6]

Over the past 5 years, experiments were suggestive of the fact that, stressors reduced the fertility rate by interfering the follicular phase of estrous cycle. Acute stressors like restraint, transport, heat, electric foot shock and hypoglycemia lead to activation of hypothalamus pituitary adrenal axis. It was predicted that, the pulsatility of GnRHrLH was slow and inadequate for the maturation of growing follicles which required faster pulse frequencies. Thus, the animal was unable to maintain estrous cycles and anestrous phase occurred.[16.]

From the analogy of the facts, it can be concluded that stress results in the reduction of primary, secondary and Graafian follicular count with the decline in the oocyte quality as well as reproductive performance of female mice.

CONCLUSION

Hunger stress affects the ovaries by reducing its weight and number of follicles.

AUTHORS' CONTRIBUTIONS

MY conducted the research. HS drafted and wrote the article. NW helped in tabulation, reference writing and critical revision of final article.

REFERENCES

1. Słuczanowska-Głąbowska S, Laszczyńska M, Piotrowska K, Głąbowski W, Rumianowski B, Masternak M, *et al*. The effect of calorie restriction on the presence of apoptotic ovarian cells in normal wild type mice and low-plasma-IGF-1 Laron dwarf mice. J Ovarian Res 2013;6(1):67.
2. Al-Qudah MM. The histological effect of hunger stress on the stomach in male albino rats: a study of light microscope. Res J Biol Sci 2011;6(11):569–74.
3. Bitgul G, Tekmen I, Keles D, Oktay G. Protective effects of resveratrol against chronic immobilization stress on testis. ISRN Urol 2013;2013:278720.
4. Hamrick MW, Ding KH, Ponnala S, Ferrari SL, Isales CM. Caloric restriction decreases cortical bone mass but spares trabecular bone in the mouse skeleton: implications for the regulation of bone mass by body weight. J Bone Miner Res 2008;23(6):870–8.
5. Faldikova L, Diblikova I, Canderle J, Zraly Z, Veznik Z, Sulcova A. Effects of nutrition, social factors and chronic stress on the mouse Leydig cell testosterone production. Vet Med-PRAHA 2001;46(6):160–8.
6. Webb R, Garnsworthy PC, Gong JG, Armstrong DG. Control of follicular growth: local interactions and nutritional influences. J Anim Sci 2004;82(13 Suppl):E63–74.
7. Lei Y, Chen J. Inhibitory effects of various types of stress on gastric tone and gastric myoelectrical activity in dogs. Scand J Gastroenterol 2009;44(5):557–63.
8. Nakamura K, Sheps S, Arck PC. Stress and reproductive failure: past notions, present insights and future directions. J Assist Reprod Genet 2008;25(2-3):47–62.
9. Waseem N, Butt SA, Hamid S. Amelioration of lead induced changes in ovary of mice, by garlic extract. J Pak Med Assoc 2014;64(7):798–801.
10. Jensen F, Willis MA, Leopardo NP, Espinosa MB, Vitullo AD. The ovary of the gestating South American plains vizcacha (Lagostomus maximus): suppressed apoptosis and corpora lutea persistence. Biol Reprod 2008;79(2):240–6.
11. Saraswathi C, Sreemantula S, Prakash WS. Effect of chronic cold restraint and immobilization stress on estrous cycle in rats. Pharmacol Online 2010;2(3):151–60.

12. Roth Z, Meidan R, Braw-Tal R, Wolfenson D. Immediate and delayed effects of heat stress on follicular development and its association with plasma FSH and inhibin concentration in cows. J Reprod Fertil 2000;120(1):83–90.
13. Shimizu T, Ohshima I, Ozawa M, Takahashi S, Tajima A, Shiota M, *et al*. Heat stress diminishes gonadotropin receptor expression and enhances susceptibility to apoptosis of rat granulosa cells. Reproduction 2005;129(4):463–72.
14. Dorfman M, Arancibia S, Fiedler JL, Lara HE. Chronic intermittent cold stress activates ovarian sympathetic nerves and modifies ovarian follicular development in the rat. Biol Reprod 2003;68(6):2038–43.
15. McGee EA, Hsueh AJ. Initial and cyclic recruitment of ovarian follicles. Endocr Rev 2000;21(2):200–14.
16. Dobson H, Smith RF. What is stress, and how does it affect reproduction? Anim Reprod Sci 2000;60:743–52.

Received: 27 June, 2017	*Revised: --*	*Accepted: 5 October, 2017*

Address for Correspondence:

Hina Siddiqi, Department of Anatomy, Pak International Medical College, Peshawar-Pakistan
Cell: +92 334 553 5906
Email: siddiqihina@yahoo.com